Kidney International, Vol. 44 (1993), pp. 1366–1371

Free and protein-bound tryptophan in serum of untreated patients with chronic renal failure

MACKENZIE WALSER and SYLVIA B. HILL

Department of Pharmacology and Molecular Sciences and Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Free and protein-bound tryptophan in serum of untreated patients with chronic renal failure. In fasting sera from 46 untreated patients with chronic renal failure and in 22 normal subjects, non-protein-bound tryptophan, F, was separated by pH-controlled equilibrium dialysis. Total tryptophan, T, and F were measured by HPLC. Results in patients were related to gender, severity of chronic renal failure (as measured by radioisotopic glomerular filtration rate), protein intake (as measured by 24-hr urinary urea N excretion), and protein nutrition (as measured by serum concentrations of albumin and transferrin). T was subnormal in 59% of the patients. In seven hypoalbuminemic patients, F/T was markedly increased (approaching unity) but F was normal. In 39 non-hypoalbuminemic patients, F was again normal but F/T was often increased at glomerular filtration rates below 30 ml/min/3 m^2 of height^2, especially in females. T was significantly correlated with estimated protein intake ($r = 0.54$, $P = 0.0004$), even though neither F nor serum protein levels were correlated with it. We conclude that the serum level of free tryptophan is well-maintained in chronic renal failure, being uninfluenced by severity of renal insufficiency, voluntary protein intake, or serum protein concentrations. On the other hand, protein-bound tryptophan varies with protein intake, decreases markedly in hypoalbuminemic patients, and also decreases in many non-hypoalbuminemic patients (especially females) when the glomerular filtration rate falls below approximately 30 ml/min/3 m^2 of height^2.

Tryptophan, unlike other amino acids in plasma, is largely protein-bound. Assessment of the effective circulating concentration of tryptophan therefore requires the determination of non-protein-bound tryptophan.

In chronic renal failure, total serum or plasma tryptophan is generally reduced owing to displacement of protein-bound tryptophan by other ligands that accumulate [1–5]. Other aspects of tryptophan levels in chronic renal failure are unclear. For example, free (that is, non-protein-bound) tryptophan in patients with uremia is reported to be high [3, 4, 6], normal [7], or low [8]. In cerebrospinal fluid of pre-dialysis patients, tryptophan is increased [7, 9]. In muscle of patients on continuous peritoneal dialysis, tryptophan levels may be high even though plasma free tryptophan is low, according to a preliminary report [10].

In chronically uremic rats, total plasma tryptophan is also low [11–14] but free tryptophan is high [13, 14], especially when dietary protein is restricted [10]. Tryptophanuria is prominent [11]. Brain levels of indoles may be high.

Many indolic metabolites accumulate in uremic plasma [4–6, 15–19]. Some are known carcinogens [15], and others have major neuropsychiatric effects [20]. Until recently, studies of the neuropsychiatric effects of tryptophan and its metabolites focused principally on the 5-hydroxytryptamine pathway. More recently, metabolites in the kynurenine pathway, which accounts for 95% of tryptophan catabolism [21], have attracted attention. Quinolinic acid, for example, is a potent neurotoxin, the formation of which depends on tryptophan intake. Involvement of these metabolites in many psychiatric disorders has been postulated [22].

The possibility that tryptophan may play a role in the progression of chronic renal failure is suggested by the observation that tryptophan radicals are readily generated by one-electron oxidation [23, 24]. However, tryptophan and some of its metabolites are effective antioxidants [25]. The importance of oxygen free radicals in the progression of renal injury has recently been stressed [26, 27]. We have reported that serum free tryptophan level is significantly correlated with rate of progression of chronic renal failure [28], that is, higher levels are associated with faster progression, as measured by rate of decline of glomerular filtration rate.

The purpose of the present study was to assess free and total tryptophan levels in untreated pre-dialysis patients.

Methods

Fasting serum (or, in preliminary studies, plasma) was obtained from 46 patients with chronic renal failure and 22 normal subjects. In patients, but not in normal subjects, whole blood was also obtained, chilled in ice, and pH was measured at 25° within 30 minutes.

Determination of free and bound tryptophan in plasma or serum

The following method was modified from Rocchi et al [29].

Venous blood was drawn with minimal stasis into a 5 or 10 ml heparinized syringe, which was immediately placed in ice. Within one hour pH was measured at room temperature. Another tube of blood was drawn and allowed to clot.

Equilibrium dialysate was prepared as follows: 0.3 ml of 6% dextran in 0.9% NaCl, adjusted to pH 7.4, was placed in a Visking tube 8/32 cellulose dialysis bag. The bag was immersed

Received for publication December 14, 1992
and in revised form July 15, 1993
Accepted for publication July 15, 1993

in 3 ml plasma or serum which had been readjusted to the original blood pH, and covered with mineral oil. Dialysis was continued overnight in the dark. For total tryptophan, 1 ml of plasma or serum was mixed with 0.2 ml of 30% trichloroacetic acid and centrifuged at 2000 g for five minutes. The supernatant was filtered through 0.45 μm Millipore filter (HAWP) and stored at 4°C. Tryptophan was measured in dialysate and in protein-free supernatant of plasma or serum as follows. A Beckman Model 332 liquid chromatograph equipped with a C-18 Ultrasphere OCS 5 column, 250 mm × 4.6 mm, was used. Optical density at 278 nm was recorded and integrated. The mobile phase was 0.02 M sodium phosphate buffer, pH 5.9:methanol (HPLC-grade), 85:15. Flow rate was 1.2 ml/min. Sample size was 20 μl. Tryptophan emerged at 7 to 9 minutes. Results were calculated from peak height, not area, using a standard curve. This was done because indoxyl sulfate was found to emerge less than one minute later. In order to determine whether the presence of indoxyl sulfate in these samples increased the height of the tryptophan peak, samples from 14 patients were rerun using a mobile phase consisting of acetic acid 0.2 M:isopropanol:tetrahydrofuran, 84:10:6, adjusted to pH 6.4 with triethylamine [30]. In this system, tryptophan emerged at 3.9 minutes and indoxyl sulfate emerged at 6.0 minutes. Tryptophan concentrations estimated by this alternative method were 14 ± 4% (SEM) higher; indoxyl sulfate concentrations are to be reported elsewhere. Thus, no substantial error was introduced by the presence of indoxyl sulfate.

In order to determine whether other compounds coelute with tryptophan, samples from 10 of these patients were incubated with tryptophanase (Sigma Chemicals) for 10 minutes at 37° in the presence of pyridoxal phosphate using phosphate buffer pH 8.3 [31]. The tryptophan peak disappeared (indoxyl sulfate is also destroyed by this enzyme).

Other observations on this method

We have found that: (1) recovery of tryptophan added to normal or uremic serum averages 98 ± 1% (SEM) (N = 8); (2) protein binding is pH-dependent, particularly in the acid range (Fig. 1); (3) free tryptophan is stable at 4°C for at least two weeks in plasma or serum stored at 4°C; (4) heparinized plasma contains 3 ± 1 (SEM) % less tryptophan per unit volume than serum (this is probably a dilutional effect); (5) free tryptophan is 9 ± 3 (SEM) % higher in plasma than in serum; thus, protein-binding is only slightly reduced by heparin, contrary to Stefanini and Biggio [32] but in confirmation of Bauman and Perry [33].

Determination of glomerular filtration rate in patients

Following an oral water load of 10 to 15 ml/kg, 100 μCi of ^{99m}Tc-labeled diethylenetriamine-pentaacetic acid (DTPA) was injected i.v. Beginning one hour later, three timed urine collections of 30 to 40 minutes with blood samples at the beginning and end of each collection were obtained. Urinary clearance of the isotope was calculated for each period, averaged, and expressed per 3 m^2 of height^2 (because average normal height^2 is approximately 3 m^2). Height rather than surface area was used as a referent because it does not change, whereas weight does, leading to misleading inferences in some patients [28].

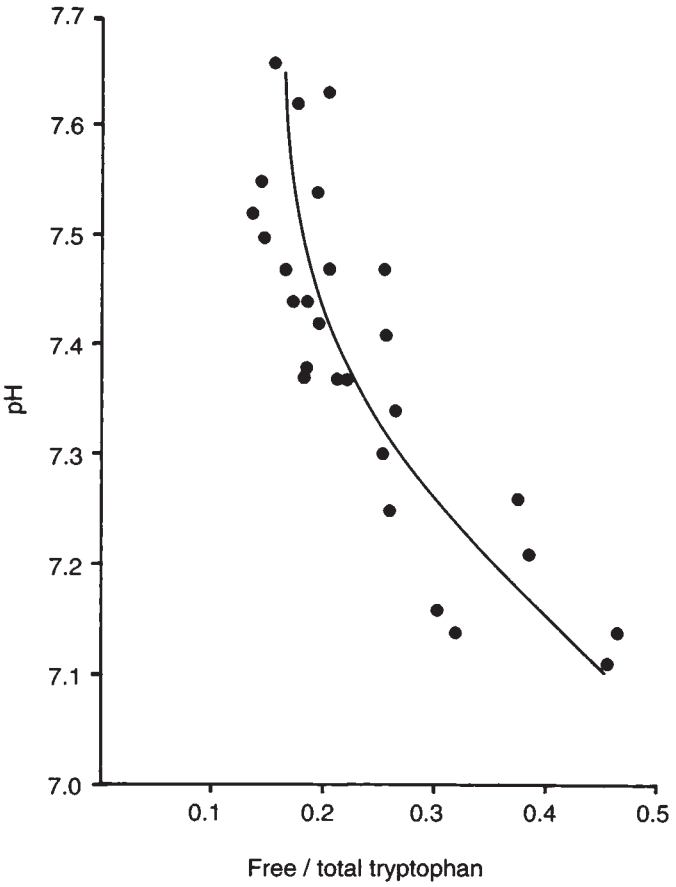

Fig. 1. *pH dependence of protein-binding of tryptophan.* A pooled sample of normal serum was dialyzed repeatedly, after modifying its pH by addition of Na_2HPO_4 or NaH_2PO_4.

Estimation of protein intake in patients

Twenty-four hour urinary excretion rate of urea N (UNA) was measured. Protein intake, in g/day, was estimated as 6.25 (UNA + 0.031 wt), where wt is weight in kg [34]. These calculations are based on the assumptions that these untreated patients were in N balance and had unchanging serum urea N concentration.

Other measurements in patients were performed in the Clinical Chemistry Laboratory.

Results

Hypoalbuminemic patients

Seven of 46 patients exhibited hypoalbuminemia (serum albumin ≤ 3.4 g/dl). Their data were considered separately and are displayed in detail in Table 1, in order of increasing serum albumin concentration. Proteinuria in this group averaged 9 g/day. Serum transferrin was subnormal in five. Mean free tryptophan level, F, 11.8 ± 4.4 μM, was not different from normal subjects (Table 2), but total tryptophan, T, and protein-bound tryptophan were greatly reduced. In the most severely hypoalbuminemic patient (#71), the ratio, F/T, of free to total tryptophan, 1.07, was the ratio to be expected if protein-binding were absent (because serum is approximately 93% water). In all seven, protein-bound tryptophan, B, calculated as B = (1.07 T)

Table 1. Observations in seven hypoalbuminemic patients

Pt #	Age *years*	Sex	Dx	EPI *g/day*	U_{Prot} *g/day*	GFR *ml/min*	Alb *g/dl*	Trans *mg/dl*	Chol *mg/dl*	TG *mg/dl*	Tryptophan Total *μM*	Tryptophan Free *μM*	Free/total
71	39	F	G	73	24	28	2.3	185	443	153	18.5	19.9	1.07
118	64	M	H	40	5	4	2.5	169	292	311	14.3	6.8	0.48
44	31	M	D	32	5	9	2.6	162	201	62	21.4	9.7	0.45
46	80	F	G	39	6	31	3.1	234	384	177	25.3	14.3	0.56
61	37	M	D	93	5	41	3.1	223	181	55	29.6	12.8	0.45
60	72	F	F	44	3	26	3.2	183	273	148	15.2	11.5	0.76
65	37	M	D	51	14	20	3.2	214	340	100	16.1	7.8	0.49
Mean	45		53		9	23	2.9	196	302	144	20.0	11.8	0.61
SD	26			22	8	13	0.4	28	94	87	5.7	4.4	0.23

Abbreviations are: EPI, estimated protein intake; U_{Prot}, proteinuria; GFR, glomerular filtration rate (corrected to 3 m^2 of $height^2$); Alb, serum albumin; Trans, serum transferrin; Chol, serum cholesterol; TG, serum triglycerides; Dx, diagnosis—D, insulin-dependent diabetes; F, fibrillary nephritis; G, glomerulonephritis; H, arteriolar nephrosclerosis.

Table 2. Free and total tryptophan in serum of non-hypoalbuminemic chronic renal failure patients, compared with normal subjects

	N	Total trp, *μM*	Free trp, *μM*	Free/total
Females				
Normals	10	50.4 ± 8.2	11.0 ± 3.2	0.21 ± 0.04
Patients	13	30.3 ± 6.0	10.4 ± 2.5	0.36 ± 0.12
P		0.0001	NS	0.0012
Males				
Normals	12	50.5 ± 6.4	10.6 ± 3.0	0.21 ± 0.06
Patients	26	37.6 ± 7.5	9.2 ± 2.0	0.25 ± 0.06
P		0.0001	NS	0.055

Data are means ± SD.

Table 3. Correlation coefficients between measured parameters in 39 non-hypoalbuminemic patients

	GFR	Alb	Trans	EPI
Total trp	0.35[a]	NS	NS	0.54[c]
Free trp	−0.40[b]	NS	NS	NS
Free/total	−0.44[b]	NS	NS	NS
GFR	—	NS	NS	NS
Alb		—	NS	NS
Trans			—	NS

Abbreviations are: trp, tryptophan; GFR, glomerular filtration rate; Alb, serum albumin; Trans, serum transferrin; EPI, estimated protein intake.

[a] $P < 0.05$

[b] $P < 0.02$

[c] $P = 0.0004$

−F, was more reduced than was serum albumin concentration. The explanation for this apparent reduction in the affinity of albumin for binding of tryptophan is not apparent, although some reduction is attributable to renal failure and protein restriction (see below).

Non-hypoalbuminemic patients

As shown in Table 2, T was considerably reduced (especially in females), compared to normal subjects. F, however, was normal. T was below the 95% confidence limit of normal (34 *μM*) in half of these non-hypoalbuminemic patients. Thus the ratio F/T was increased, particularly in females.

Within this group of 39 patients we sought to determine which factors were important determinants of F and T. Age was not a significant predictor, nor was serum level of transferrin or albumin (although, as indicated previously, albumin is a major determinant when hypoalbuminemic patients are also considered). As shown in Table 3 and Figure 2, protein-binding was significantly correlated (negatively) with glomerular filtration rate. T was positively correlated with estimated protein intake (Fig. 3), which varied from 25 to 114 g/day (mean 63 ± 22 SD g/day), but F was not (Fig. 4).

When stepwise multiple regression was used to determine which of the measured independent variables were predictors of B, we found that estimated protein intake, glomerular filtration rate and gender were all significant predictors. The combined regression equation is:

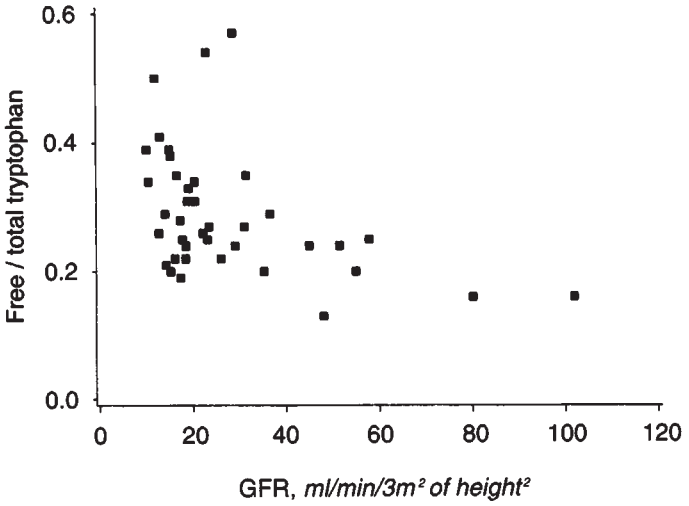

Fig. 2. *Ratio of free to total tryptophan in serum of 39 non-hypoalbuminemic patients as a function of glomerular filtration rate.*

$B = 1.0 \pm 3.4 + (0.124 \pm 0.054)\ EPI + (0.163 \pm 0.054)\ GFR + (5.6 \pm 2.5)$ Gender, where Gender = 0 for females and 1 for males.

This equation predicts 48% (= r^2) of the observed variation in B in these 39 non-hypoalbuminemic patients.

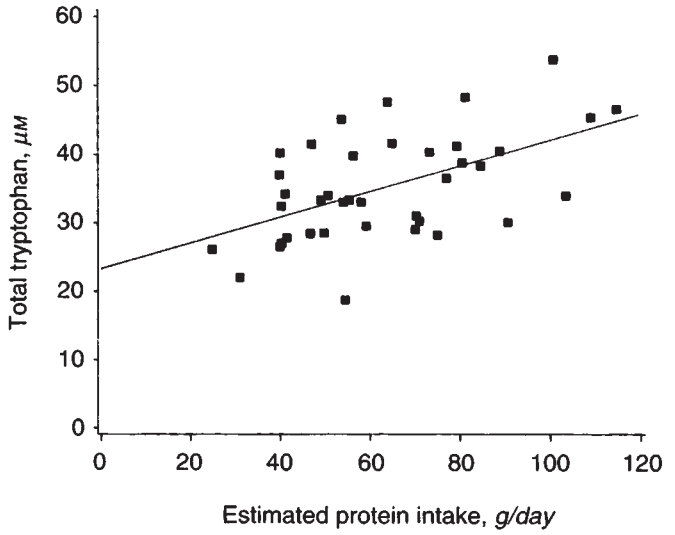

Fig. 3. *Total serum tryptophan as a function of estimated protein intake in the same samples as in Figure 2.* Linear regression shown.

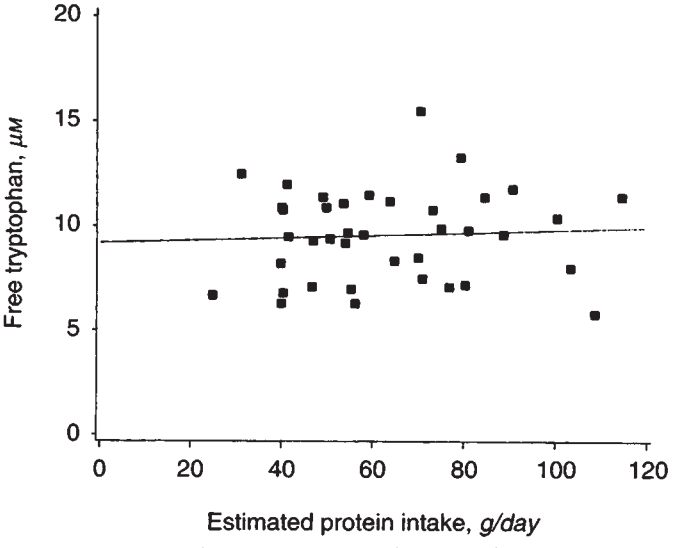

Fig. 4. *Free tryptophan in serum as a function of estimated protein intake in the same samples as in Figure 2.* Linear regression shown.

Discussion

We have identified fifteen previous studies in which mean values for T and F in normal human plasma or serum are reported (Table 4). Our results in normal subjects are comparable to these previous data.

The two methods used for measuring F, ultrafiltration and equilibrium dialysis, both have their disadvantages. pH control during ultrafiltration is difficult to achieve, even when it is carried out in a CO_2-containing atmosphere. The progressive rise in plasma or serum pH that usually occurs during ultrafiltration in air is probably responsible for some of the lower values for free/total tryptophan that have been reported (Table 4). Equilibrium dialysis necessarily disturbs the equilibrium between free and bound tryptophan. The extent of the error so introduced has been considered by Chadwick, Phipps and Powell [35]. In the present method, the ratio of volume of serum to volume of dialysate is 10:1, so that little change in this equilibrium is expected.

Table 4. Mean free and total tryptophan in normal human plasma or serum, μmol/liter, according to various workers

Method	Total	Free	Free/total	Reference
Ultrafiltration	68	5	0.07	48
	68	9	0.14	33
	70	10	0.12	8
	57	2	0.04	49[a]
	56	12	0.21	50, 51
	71	7	0.10	6
	68	4	0.07	4
	52	8	0.15	7
	68	18	0.26	52
Equilibrium dialysis	84	9	0.12	53
	66	6	0.09	54
	51	12	0.24	10
	69	17	0.24	55
	42	9	0.22	29
	66	8	0.12	30
	50	11	0.21	This study

[a] Children

In renal failure, the importance of pH control increases, since acidosis is often present. None of our patients had severe acidosis. Nevertheless, we felt it was important to maintain serum pH at its *in vivo* value during dialysis.

Comparison of our findings in chronic renal failure patients with the previous studies that we have identified [3, 4, 6–8] shows that our results are more similar to those of Sullivan et al [7] than to the others. Sullivan et al [7] and Lagana et al [6] did not control pH during ultrafiltration. Sullivan et al [7] corrected measured values of ultrafiltrate tryptophan for measured pH of stored plasma using a standard curve. Saito et al [4] adjusted serum pH to 7.4 before ultrafiltration but did not measure pH after ultrafiltration. These patients were all on dialysis, in contrast to our study and that of Sullivan et al [7] and Cernacek et al [8]. Lagana et al [6] do not state whether or not their patients were on dialysis.

Tryptophan concentration may be important in chronic renal failure for several reasons: (1) it is an essential amino acid that is not only required for protein synthesis, but also is believed to play a unique role in the regulation of protein turnover [21, 36–41]; (2) supplements to very low protein diets currently under study in the treatment of chronic renal failure generally contain all of the essential amino acids as such or as their keto-analogues, with the exception of tryptophan, which may be low or absent; (3) administration of tryptophan may cause a severe disorder known as the eosinophilia-myalgia syndrome, which may or may not be caused by a specific contaminant [42]; (4) as noted in the introduction, tryptophan metabolites exert neuropsychiatric effects; (5) in a retrospective analysis, F was significantly correlated with rate of decline of glomerular filtration rate [28].

The present study was limited to untreated patients and therefore sheds no light on points (2), (3), or (5). However, our observation that F is well-maintained in these patients suggests neither point (1) nor point (4) are sources of concern.

The absence of any dependence of F on protein intake over a fivefold range (Fig. 4) is surprising, since fasting concentrations

of other essential amino acids are generally strongly dependent on the level of protein intake [43–46]. These findings suggest that metabolic degradation of tryptophan, most of which occurs via the kynurenine pathway [21], may be regulated differently in response to variations in protein intake than are the degradative pathways of other essential amino acids, most of which involve oxidative enzymes.

The strong dependence of T on protein intake (Fig. 3) is also surprising, particularly because there was no correlation between serum albumin concentration and protein intake (Table 3). Thus, the apparent affinity of albumin for binding of tryptophan increases with protein intake, and conversely, if interference by organic acids (including free fatty acids) in the albumin-binding of tryptophan is invoked to explain these results, the responsible organic acids would necessarily be present at higher levels in low protein diets. This seems implausible. However, a change in as yet unidentified plasma constituents that increase or decrease the affinity of albumin for tryptophan may explain these findings. It would be of interest to vary protein intake and determine whether bound tryptophan changes.

The diminution in protein-binding of tryptophan at low levels of GFR (Fig. 2) is almost certainly attributable to competition from organic acids, derived from metabolism and retained owing to impaired tubular secretion, as has been pointed out previously [1–5].

In hypoalbuminemic subjects (Table 1), a further decrease in albumin affinity for tryptophan is apparent, not explained by their protein intake or severity of renal failure. Conceivably, hyperlipidemia, which was present in most of these subjects (Table 1) may be responsible. Cholesterol level was correlated with F/T in this small series ($r = 0.68$) but triglyceride level was not. We did not measure levels of free fatty acids, which may be particularly effective in competing for binding of tryptophan [47].

Acknowledgments

This study supported by Clinical Center Grant RR-00722 from the National Institutes of Health.

Reprint requests to Mackenzie Walser, M.D., Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore, Maryland 21205, USA

References

1. Depner TA, Gulyassy PF: Plasma protein binding in uremia: Extraction and characterization of an inhibitor. *Kidney Int* 18:86–94, 1980
2. de Torrente A, Glazer GB, Gulyassy P: Reduced *in vitro* binding of tryptophan by plasma in uremia. *Kidney Int* 6:222–229, 1974
3. Gulyassy PF, Peters JH, Schoenfeld G: Transport and protein binding of tryptophan in uraemia, in *Uraemia*, edited by Kluthe R, Berlyne G, Burton B, Stuttgart, Georg Thieme Verlag, 1972, pp. 163–177
4. Saito S, Niwa T, Maeda K, Kobayashi K, Yamamoto Y, Ohta K: Tryptophan and indolic tryptophan metabolites in chronic renal failure. *Am J Clin Nutr* 33:1402–1406, 1980
5. Mabuchi H, Nakahashi H: Isolation and characterization of an endogenous drug-binding inhibitor present in uremic serum. *Nephron* 44:277–281, 1986
6. Lagana A, Liberti A, Morgia C, Tarola AM: Determination of indican and tryptophan in normal and uraemic patients by high-performance liquid chromatography with a new electrochemical detector. *J Chromatog* 378:85–93, 1986
7. Sullivan PA, Murnaghan D, Callaghan N, Kantamaneni BD, Curzon G: Cerebral transmitter precursors and metabolites in advanced renal disease. *J Neurol Neurosurg Psychiatry* 41:581–588, 1978
8. Cernacek P, Becvarova H, Gerova Z, Valek A, Spustova V: Plasma tryptophan level in chronic renal failure. *Clin Nephrol* 14:246–249, 1980
9. Sperscheider H, Spustova V, Stein G, Dzurik R: Middle molecular weight substances in the cerebrospinal fluid of uremic patients. *Clin Nephrol* 17:298–302, 1982
10. Lindholm B, Garcia E, Qureshi GA, Henriksson S, Bergström J: High muscle and low plasma tryptophan in CAPD. *Kidney Int* 36(Suppl 27):S–302, 1989
11. Holmes EW, Kahn SE: Tryptophan distribution and metabolism in experimental chronic renal insufficiency. *Exp Mol Pathol* 46:89–101, 1987
12. Mannan A, Wiebe LI, Noujaim AA, Secord DC: Plasma free amino acids in the chronically uremic rat. *Clin Biochem* 8:303–306, 1975
13. Siassi F, Wang M, Kopple JD, Swendseid ME: Brain serotonin turnover in chronically uremic rats. *Am J Physiol* 232:E526–E528, 1977
14. Siassi F, Wang M, Kopple JD, Swendseid ME: Plasma tryptophan levels and brain serotonin metabolism in chronically uremic rats. *J Nutr* 107:840–845, 1977
15. Manabe S, Yanagisawa H, Guo SB, Abe S, Ishikawa S, Wada O: Detection of Trp-P-1 and Trp-P-2, carcinogenic tryptophan pyrolysis products, in dialysis fluid of patients with uremia. *Mutat Res* 179:33–40, 1987
16. Manabe S, Suzuki M, Kusano E, Wada O, Asano Y: Elevation of levels of carcinogenic tryptophan pyrolysis products in plasma and red blood cells of patients with uremia. *Clin Nephrol* 37:28–33, 1992
17. Tavares-Almeida I, Gulyassy PF, Depner TA, Jarrard EA: Aromatic amino acid metabolites as potential protein binding inhibitors in human uremic plasma. *Biochem Pharmacol* 34:2431–2438, 1985
18. Swan JS, Kragten EY, Veening H: Liquid-chromatographic study of fluorescent materials in uremic fluids. *Clin Chem* 29:1082–1084, 1983
19. Byrd DJ, Berthold HW, Trefz KF, Kochen W, Gilli G, Schärer K, Schüler HW, Asbach HW: Indolic tryptophan metabolism in uraemia. *Proc Eur Dial Transplant Assoc* 12:347–354, 1976
20. Freese A, Swartz KJ, During MJ, Martin JB: Kynurenine metabolites of tryptophan: Implications for neurologic diseases. *Neurology* 40:691–695, 1990
21. Peters JC: Tryptophan nutrition and metabolism. in *Kynurenine and Serotonin Pathways—Progress in Tryptophan Research* (vol 294), edited by Schwarcz, R, Young SN, Brown RR, New York, Plenum Press, 1991, pp. 345–358
22. Schwarcz R, Du F: Quinolinic acid and kynurenic acid in the mammalian brain, in *Kynurenine and Serotonin Pathways—Progress in Tryptophan Research* (vol 294), edited by Schwarcz R, Young SN, Brown RR, New York, Plenum Press, 1991 pp. 185–199
23. Hoey BM, Butler J: The repair of oxidized amino acids by antioxidants. *Biochim Biophys Acta* 791:212–218, 1984
24. Bisby RH, Ahmed S, Cundall RB: Repair of amino acid radicals by a vitamin E analogue. *Biochem Biophys Res Commun* 119:245–251, 1984
25. Christen S, Peterhans E, Stocker R: Antioxidant activities of some tryptophan metabolites: Possible implication for inflammatory diseases. *Proc Natl Acad Sci USA* 87:2506–2510, 1990
26. Shah SV: Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int* 35:1093–1106, 1989
27. Salahudeen AK, Clark EC, Nath KA: Hydrogen peroxide-induced renal injury: A protective role for pyruvate *in vitro* and *in vivo*. *J Clin Invest* 88:1886–1893, 1991
28. Walser M: Progression of chronic renal failure in man. *Kidney Int* 37:1195–1210, 1990
29. Rocchi E, Farina F, Silingardi M, Cassalgrandi G, Gaetani

E, LAURERE CF: High performance liquid chromatographic determination of total and free tryptophan in serum from control subjects and liver patients. *J Chromatog* 380:128–132, 1986
30. NIWA T, TAKEDA N, TAKEMATSU A, MAEDA K: Accumulation of indoxyl sulfate, an inhibitor of drug-binding, in uremic serum as demonstrated by internal-surface reversed-phase liquid chromatography. *Clin Chem* 34:2264–2267, 1988
31. COLOWICK SP, KAPLAN NO: *Methods in Enzymology* (vol II), New York, Academic Press, 1955, p. 238
32. STEFANINI E, BIGGIO G: A simple method for determination of free tryptophan in serum. *Rivisita Farmacologia Terapia* 11:49–51, 1975
33. BAUMAN P, PERRY M: The analysis of free tryptophan in human blood with the ultrafiltrator: A comparison with other methods. *Clin Chim Acta* 76:223–231, 1977
34. MARONI BJ, STEINMAN TI, MITCH WE: A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 27:58–65, 1985
35. CHADWICK C, PHIPPS DA, POWELL C: Potential methodological errors in the application of equilibrium dialysis to the determination of "free" and "bound" plasma tryptophan. *Clin Chim Acta* 99:179–282, 1979
36. MUNRO HN: Regulation of mammalian protein synthesis. *Fed Proc* 27:1231–1235, 1968
37. WOODSIDE KH, MORTIMORE GE: Suppression of protein turnover by amino acids in the perfused rat liver. *J Biol Chem* 247:6474–6481, 1972
38. LARDEUX BR, MORTIMORE GE: Amino acid and hormonal control of macromolecular turnover in perfused rat liver. *J Biol Chem* 262:14514–14519, 1987
39. LIN FD, SMITH TK, BAYLEY HS: A role for tryptophan in regulation of protein synthesis in porcine muscle. *J Nutr* 118:445–459, 1988
40. CORTIMARA NO, SEVE B, LEBRETON Y, GANIER P: Effect of dietary tryptophan on muscle, liver and whole-body protein synthesis in weaned piglets: Relationship to plasma insulin. *Br J Nutr* 66:423–435, 1991
41. SIDRANSKY H, VERNEY E, MURTY CN: Studies on the influence of tryptophan and related compounds on hepatic polyribosomes and protein synthesis in the rat. *J Nutr* 110:2231–2242, 1980
42. CLAUW DJ, NASHEL DJ, UMHAU A, KATZ P: Tryptophan-associated eosinophilic connective-tissue disease. *JAMA* 263:1502–1506, 1990
43. MILSOM JP, MORGAN MY, SHERLOCK S: Factors affecting plasma amino acid concentrations in control subjects. *Metabolism* 28:313–319, 1979
44. WELLER LA, MARGEN S, CALLOWAY DH, MEISSNER EF: Serum amino acids in young consuming diets differing in level and pattern of amino acids. *Am J Clin Nutr* 26:722–727, 1973
45. SWENSEID ME, YAMADA C, VINYARD E, FIGUEROA WG: Plasma amino acid levels in young subjects receiving diets containing 14 or 3.5 g nitrogen per day. *Am J Clin Nutr* 21:1381–1383, 1968
46. KOPPLE JD, COBURN JW, GORDON S, RUBINI ME: Controlled comparison of 20g and 40g protein diets in the treatment of chronic uremia. *Am J Clin Nutr* 21:553–564, 1968
47. MCMENAMY RH: Binding of indole analogues to human serum albumin. Effects of fatty acids. *J Biol Chem* 24:4235–4243, 1965
48. AYLWARD M, FOWLER PD, JOHN V, MADDOCK J, SELDRUP J: The influence of diclofenac sodium (Voltarol®) on free, protein-bound and total plasma L-tryptophan in adult healthy male subjects. *Rheumatol Rehabil Suppl* 2:47–59, 1979
49. FERGUSON HB, PAPPAS BA, TRITES RL, PETERS DAV, TAUB H: Plasma free and total tryptophan, blood serotonin, and the hyperactivity syndrome: No evidence for the serotonin deficiency hypothesis. *Biol Psychiatry* 16:231–238, 1981
50. HIJIKATA Y, HARA K, SHIOZAKI Y, MURATA K, SAMESHIMA Y: Determination of free tryptophan in plasma and its clinical applications. *J Clin Chem Clin Biochem* 22:291–299, 1984
51. HIJIKATA Y, HARA K, EGAWA H, MIZUMO T, SHIOZAKI Y, MURATA K, SAMESHIMA Y: Microdetermination of unbound tryptophan in plasma by a combination of ultrafiltration and high performance liquid chromatography. *Anal Biochem* 118:10–16, 1981
52. BECK O, HESSELGREN T: Method for the determination of tryptophan in serum and cerebrospinal fluid. *J Chromatogr* 181:100–102, 1980
53. BENDER DA, BAMJI AN: Serum tryptophan binding in chlorpromazine-treated chronic schizophrenics. *J Neurochem* 22:804–809, 1974
54. LIBONDI T, RINALDI E, MIELE L, MENZIONE M, PULCINO D, DELLA PIETRA F, AURICCHIO G: Serum tryptophan in humans with senile cataracts. *Metab Pediatr Systemic Ophthal* 7:75–79, 1983
55. LIPSETT D, MADRAS BK, WURTMAN RJ, MUNRO HN: Serum tryptophan level after carbohydrate ingestion: Selective decline in non-albumin-bound tryptophan coincident with reduction in serum free fatty acids. *Life Sci* 12 (part II):57–64, 1973